Applicant	Focus Diagnostics, Inc. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Tara Viviani tel 562.240.6115 fax 562.240.6530 tviviani@focusdx.com
Summary Date	May 18, 2012
Proprietary Name	Simplexa™ Flu A/B & RSV Direct Simplexa™ Flu A/B & RSV Positive Control Pack
Generic Name	Respiratory Viral Panel
Classification	Class II, Special Controls
Regulation	§ 21 CFR 866.3980 – respiratory viral panel multiplex nucleic acid assay
Product Code	OCC – respiratory virus panel nucleic acid assay system
Predicate Devices	Prodesse, ProFlu+ Assay (K092500, K081030, K073029)

JUL 13 2012

INTENDED USE

The Focus Diagnostics Simplexa™ Flu A/B & RSV Direct assay is intended for use on the 3M Integrated Cycler instrument for the *in vitro* qualitative detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs (NPS) from human patients with signs and symptoms of respiratory tract infection in conjunction with clinical and epidemiological risk factors. This test is intended for use as an aid in the differential diagnosis of influenza A, influenza B, and RSV viral infections in humans and is not intended to detect influenza C.

Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A were established with clinical specimens collected during the 2010/2011 influenza season when 2009 H1N1 influenza and H3N2 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to the state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

INTENDED USE

Focus Diagnostics' Simplexa™ Flu A/B & RSV Positive Control Pack is intended to be used as a control with the Simplexa™ Flu A/B & RSV Direct kit. This control is not intended for use with other assays or systems.

Device Description

The Simplexa™ Flu A/B & RSV Direct assay system is a real-time RT-PCR system that enables the direct amplification, detection and differentiation of human influenza A (Flu A) virus RNA, human influenza B (Flu B) virus RNA and RSV RNA from unprocessed nasopharyngeal swabs that have not undergone nucleic acid extraction. The system consists of the Simplexa™ Flu A/B & RSV Direct assay, the 3M Integrated Cycler (with Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories.

In the Simplexa™ Flu A/B & RSV Direct assay, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify Flu A, Flu B, RSV and internal control RNA. The assay provides three results; conserved regions of influenza A viruses (matrix gene), influenza B viruses (matrix gene) and RSV (M gene)

are targeted to identify these viruses in the specimen. An RNA internal control is used to detect RT-PCR failure and/or inhibition.

The 3M Integrated Cycler is a rapid real-time Polymerase Chain Reaction thermocycler used for the identification of nucleic acid from prepared biological samples. The instrument utilizes disk media to contain and to process samples. The instrument uses real time flourometric detection to identify targets within the sample wells. The instrument is controlled by an external computer running the Integrated Cycler Studio software.

Predicate Device Information

Trade Name / Method	510(k) submitter	510(k) number	Decision Date	Panel	Product Code(s)
ProFlu+	GenProbe (Prodesse)	K092500, K081030, K073029	08/20/2009 05/02/2008 01/04/2008	Microbiology (83)	OCC

Comparison to Predicate Device

Item	Device	Predicate
Name	Simplexa™ Flu A/B & RSV Direct	ProFlu+
Intended Use	The Focus Diagnostics Simplexa™ Flu A/B & RSV Direct assay is intended for use on the 3M Integrated Cycler instrument for the in vitro qualitative detection and discrimination of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs (NPS) from human patients with signs and symptoms of respiratory tract infection in conjunction with clinical and epidemiological risk factors. This test is intended for use as an aid in the differential diagnosis of influenza A, influenza B, and RSV viral infections in humans and is not intended to detect influenza C. Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Performance characteristics for influenza A were established with clinical specimens collected during the 2010/2011 influenza season when 2009 H1N1 influenza and H3N2 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.	The ProFlu™+ Assay is a multiplex Real-Time PCR (RT-PCR) in vitro diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B and RSV viral infections in humans and is not intended to detect Influenza C. Negative results do not preclude influenza or RSV virus infection and should not be used as the sole basis for treatment or other management decisions. It is recommended that negative RSV results be confirmed by culture. Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza

Item Name	Device Simplexa™ Flu A/B & RSV Direct	Predicate ProFlu+
	If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
Assay Targets	Influenza A, Influenza B, RSV	Influenza A, Influenza B, RSV
Sample Types	NPS	NPS
Extraction Methods	None	Roche MagNA Pure LC Total Nucleic Acid Isolation Kit, Biomérieux NucliSENS easyMAG
Assay Methodology	PCR-based system for detecting the presence / absence of viral RNA in clinical specimens	PCR-based system for detecting the presence / absence of viral DNA/RNA in clinical specimens
Detection Techniques	Multiplex assay using different reporter dyes for each target.	Multiplex assay using different reporter dyes for each target.
Influenza A Viral Target	Well conserved region of the matrix gene	Matrix gene
Influenza B Viral Target	Well conserved region of the matrix gene	Non-structural NS1 and NS2
Respiratory Syncytial Viral Target	M gene	Polymerase
LoD	Analytical sensitivity (LoD) as defined as the lowest concentration at which ≥ 95% of all replicates tested positive, ranges from 10^{2} – 10^{-3} $TCID_{50}$/mL.	Analytical sensitivity (LoD) as defined as the lowest concentration at which ≥ 95% of all replicates tested positive, ranges from 10^{2} – 10^{-1} $TCID_{50}$/mL.
Reproducibility	Influenza A = %CV of 0.4 to 1.5 Influenza B = %CV of 0.5 to 3.6 RSV = %CV of 1.2 to 3.3	Influenza A = %CV of 1.4 to 5.3 Influenza B = %CV of 0.7 to 3.1 RSV = %CV of 1.5 to 8.3

PERFORMANCE CHARACTERISTICS

CLINICAL AGREEMENT – PROSPECTIVE STUDY

Three external testing sites and one internal site participated in a prospective clinical study. Reference results for influenza A, influenza B viruses and respiratory syncytial virus were generated using culture. Culture results were carried forward from the results obtained at the time of sample collection. A total of 722 nasopharyngeal swabs

specimens were obtained from prospectively collected specimens from patients with signs and symptoms of viral respiratory tract infection. Prospective samples were collected in Eastern United States from 10-November-2010 to 11-March-2011; the Mid-Western United States from 08-January-2011 to 02-February 2011 and in Australia from 17-August-2010 to 20-October-2010. Of the 722 specimens 325 specimens were collected from female patients and 397 specimens were collected from male patents. A total of 327 specimens were from patients <5 years of age, 223 specimens were from patients between 5-22 years of age, 158 specimens were from patients between 22 to 60 years of age and 14 specimens were from patients >60 years of age, One (1) sample was excluded from the prospective analysis due to Invalid result for Flu A, three (3) samples were excluded due to an invalid result for Flu B and one (1) sample were excluded due to an invalid result for RSV. During the study the percentage of specimens with invalid results was 0.4% (3/722) with a 95% CI of 0.1% to 1.2%.

Clinical Agreement – Flu A (Prospective – All sites combined)

Culture Result		Simplexa™ Results - Flu A		Sensitivity/Specificity
	n	Detected	Not Detected	95% CI
Detected	68	66	2	Sensitivity: 97.1%(66/68) 95% CI: 89.9 to 99.2%
Not Detected	653	14	639	Specificity: 97.9%(639/653) 95% CI: 96.4 to 98.7%

Clinical Agreement – Flu B (Prospective – All sites combined)

Culture Result		Simplexa™ Results - FluB		Sensitivity/Specificity
	N	Detected	Not Detected	95% CI
Detected	21	21	0	Sensitivity: 100.0%(21/21) 95% CI: 84.5 to 100.0%
Not Detected	698	1	697	Specificity: 99.9%(697/698) 95% CI: 99.2 to 100.0%

Clinical Agreement – RSV (Prospective – Site 1)

Culture Result		Simplexa™ Results – RSV		Sensitivity/Specificity
	N	Detected	Not Detected	95% CI
Detected	1	1	0	Sensitivity: 100.0%(1/1) 95% CI: 20.7 to 100.0%
Not Detected	329	6[a]	323	Specificity: 98.2%(323/329) 95% CI: 96.1 to 99.2%

a) 4/6 samples were confirmed as RSV positive by an FDA cleared NAT

Clinical Agreement – RSV (Prospective – Site 2)

Culture Result		Simplexa™ Results – RSV		Sensitivity/Specificity
	N	Detected	Not Detected	95% CI
Detected	73	72	1[a]	Sensitivity: 98.6%(72/73) 95% CI: 92.6 to 99.8%
Not Detected	172	18[b]	154	Specificity: 89.5%(154/172) 95% CI: 84.1 to 93.3%

a) 1/1 sample confirmed as RSV positive by an FDA cleared DFA

b) 11/18 samples confirmed as RSV positive by an FDA cleared DFA

Clinical Agreement – RSV (Prospective – Site 3)

Culture Result		Simplexa™ Results – RSV		Sensitivity/Specificity
	N	Detected	Not Detected	95% CI
Detected	10	9	1	**Sensitivity:** 90.0%(9/10) 95% CI: 59.6 to 98.2%
Not Detected	136	21 [a]	115	**Specificity:** 84.6%(115/136) 95% CI: 77.5 to 89.7%

a) 20/21 samples confirmed as RSV positive by an FDA cleared NAT

CLINICAL AGREEMENT – RETROSPECTIVE STUDY

Three external testing sites participated in a retrospective clinical study. Reference results for influenza A, influenza B viruses and respiratory syncytial virus were generated using culture. Culture results were carried forward from the results obtained at the time of sample banking. A total of 223 nasopharyngeal swabs specimens were obtained from retrospectively banked specimens from patients with signs and symptoms of viral respiratory tract infection.

Clinical Agreement – Flu A (Retrospective – All sites combined)

Culture Result		Simplexa™ Results – Flu A		PPA/NPA*
	n	Detected	Not Detected	95% CI
Detected	79	76	3	**PPA:** 96.2% (76/79) 95% CI: 89.4 to 98.7%
Not Detected	144	1	143	**NPA:** 99.3% (143/144) 95% CI: 96.2 to 99.9%

* PPA = Positive Percent Agreement, NPA = Negative Percent Agreement

Clinical Agreement – Flu B (Retrospective – All sites combined)

Culture Result		Simplexa™ Results – Flu B		PPA/NPA*
	N	Detected	Not Detected	95% CI
Detected	41	40	1	**PPA:** 97.6% (40/41) 95% CI: 87.4 to 99.6%
Not Detected	182	0	182	**NPA:** 100.0% (182/182) 95% CI: 97.9 to 100.0%

* PPA = Positive Percent Agreement, NPA = Negative Percent Agreement

Clinical Agreement – RSV (Retrospective – All sites combined)

Culture Result		Simplexa™ Results – RSV		PPA/NPA*
	N	Detected	Not Detected	95% CI
Detected	12	12	0	**PPA:** 100.0%(12/12) 95% CI: 75.7 to 100.0%
Not Detected	211	3	208	**NPA:** 98.6%(208/211) 95% CI: 95.9 to 99.5%

* PPA = Positive Percent Agreement, NPA = Negative Percent Agreement

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) was determined for the Simplexa™ Flu A/B & RSV Direct assay using quantified stocks of influenza A, influenza B and RSV virus strains serially diluted in negative swab matrix. The lowest concentration with ≥95% detection (at least 19 out of 20 replicates) was determined to be the limit of detection for each assay.

Simplexa™ Flu A/B & RSV Direct Limit of Detection

Viral Strain	LoD ($TCID_{50}$/mL)
Influenza A/PR/8/34 (H1N1)	0.005
Influenza A/Hong Kong/8/68 (H3N2)	10
Influenza A/Swine NY/02/2009 (H1N1)	0.1
Influenza B/Great Lakes/1739/54	2
Influenza B/Malaysia/2506/2004	20
RSV A2	1
RSV B CH93-18(18)	3

ANALYTICAL REACTIVITY / CROSS REACTIVITY

Analytical Reactivity

Different strains of influenza A including H1 and H3 subtypes, influenza B and RSV including A and B subtypes were evaluated. The most recent strains and geographically diverse strains were chosen. Quantified viral material was spiked into negative swab matrix at a single dilution with a concentration of approximately 1.0 $x10^2$ or 1.0 $x10^3$ $TCID_{50}$/mL and assayed in triplicate. Ct values obtained during testing indicate all viral strains were tested near the LoD. All strains tested were appropriately detected.

Cross Reactivity (Analytical Specificity)

The Simplexa™ assay's analytical specificity was evaluated by testing the ability to exclusively identify influenza A virus and/or influenza B virus and/or RSV with no cross reactivity to organisms that are closely related, or cause similar clinical symptoms, or present as normal flora in the specimen types of interest.

The panel of thirty-two (32) potential cross reactants were individually spiked into a swab matrix at clinically relevant concentrations. The unspiked matrix was also tested to serve as a baseline. Samples were tested in triplicate to screen for cross reactivity. If signal was detected in any detection channel (Flu A, Flu B, RSV) in any of the three replicates, an additional 5 replicates were tested for confirmation.

No cross reactivity was detected for Flu A, Flu B or RSV.

INTERFERENCE

The performance of this assay was evaluated with potentially interfering substances that may be present in nasopharyngeal swabs at the concentrations indicated in the table below. The potentially interfering substances were evaluated in a contrived sample that contained influenza A (influenza A/PR/8/34 H1N1) at a concentration of 0.01 $TCID_{50}$/mL and influenza B (influenza B/Malaysia/2506/2004) at a concentration of 40 $TCID_{50}$/mL and RSV A2 at a concentration of 4 $TCID_{50}$/mL. All strains were tested at two to four times the LoD. There was no evidence of interference caused by the substances tested.

Potential Interferents	Active Ingredient	Interferent Concentration
Afrin Nasal Spray	Oxymetazoline	15% (v/v)
Antibacterial, systemic	Tobramycin	4 µg/mL
Antibiotic, nasal ointment	Mupirocin	6.6 mg/mL
Blood	N/A	2%(v/v)
Purified Mucin Protein	Bovine Submaxillary Gland Type I-S	60 µg/mL
Nasal Corticosteroid - Beconase AQ	Beclomethasone	5% (v/v)
Nasal Corticosteroid - Fluticasone	Fluticasone	5% (v/v)
Relenza Antiviral Drug	Zanamivir	3.3 mg/mL
Tamiflu Antiviral Drug	Oseltamivir	1 µM

Potential Interferents	Active Ingredient	Interferent Concentration
Zicam Nasal Gel	Luffa Opperculata, Galphimia glauca, histaminum hydrochloricum	5% (v/v)

INHIBITION BY OTHER MICROORGANISMS

The Simplexa™ assay was evaluated by testing the ability to identify influenza A virus, influenza B virus, and RSV when potentially inhibitory organisms are present.

The panel of thirty two (32) potentially inhibitory organisms was individually spiked into a pool with a low concentration (approximately 2 times LoD) of influenza A (Influenza A/PR/8/34 H1N1), influenza B (Influenza B/Malaysia/2506/2004) and RSV (A2). Samples were tested in triplicate to screen for inhibition. If signal was not detected in any detection channel (Flu A, Flu B, RSV) in any of the three replicates, an additional 5 replicates were tested for confirmation.

No inhibitory effects were confirmed for influenza A, influenza B, or RSV at the concentrations tested.

CARRY-OVER CONTAMINATION

An internal carry-over study searched for the presence of contamination in negative samples. The study was designed by alternately placing a high positive and a negative sample on each disc. The carryover effect was evaluated by comparing the observed negative rate for the negative sample with the expected rate under normal reproducibility conditions. No carry-over contamination effect was seen in the Flu A, Flu B or RSV channels.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration

10903 New Hampshire Avenue
Silver Spring, MD 20993

JUL 13 2012

Focus Diagnostics, Inc.
c/o Tara Viviani
Regulatory Affairs Project Manager
11331 Valley View Street
Cypress, California 90630

Re: K120413
Trade/Device Name: Simplexa™ Flu A/B & RSV Direct
Simplexa™ Flu A/B & RSV Positive Control Pack
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel nucleic acid assay system
Regulatory Class: Class II
Product Code: OCC, OOI
Dated: May 18, 2012
Received: May 21, 2012

Dear Ms. Viviani:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of

CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use Statement

510(k) Number (if known): k120413

Device Name: Simplexa™ Flu A/B & RSV Direct
Simplexa™ Flu A/B & RSV Positive Control Pack

Indications for Use:

Simplexa™ Flu A/B & RSV Direct (REF MOL2650)

The Focus Diagnostics Simplexa™ Flu A/B & RSV Direct assay is intended for use on the 3M Integrated Cycler instrument for the *in vitro* qualitative detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs (NPS) from human patients with signs and symptoms of respiratory tract infection in conjunction with clinical and epidemiological risk factors. This test is intended for use as an aid in the differential diagnosis of influenza A, influenza B, and RSV viral infections in humans and is not intended to detect influenza C.

Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A were established with clinical specimens collected during the 2010/2011 influenza season when 2009 H1N1 influenza and H3N2 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to the state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Simplexa™ Flu A/B & RSV Positive Control Pack (REF MOL2660)

Focus Diagnostics' Simplexa™ Flu A/B & RSV Positive Control Pack is intended to be used as a control with the Simplexa™ Flu A/B & RSV Direct kit. This control is not intended for use with other assays or systems.

Prescription Use ___X___ (Part 21 CFR 801 Subpart D) And/Or Over-The-Counter Use _____ (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Tamara Feldblyum

Division Sign-Off
Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K120413